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Publication date:
2015

Document version
Early version, also known as pre-print

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Reduced field efficacy of ivermectin against *Ostertagia ostertagi* and *Cooperia oncophora* in Danish cattle

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Field reports of anthelmintic resistance against the widely-used macrocyclic lactones (ML) in gastrointestinal nematodes (GIN) of cattle have appeared in NW-Europe in recent years.

Objective: The aim of this study was to assess the efficacy of ivermectin (IVM) against field infections with GIN in Danish cattle. In addition, we evaluated a novel quantitative (q) real-time PCR assay for accurate identification of surviving nematode species after treatment.

Methods: Six farms were selected based on mean faecal egg counts (FEC) of ≥ 100 nematode eggs per gram in first-season grazing heifers. All selected farms had a history of use of ML. In each farm, 20 heifers were selected for faecal egg count reduction test (FECRT) and individual weights were measured using a girth-tape. Ten animals were treated (s.c.) with 0.2 mg IVM/kg body weight and ten animals were left untreated as controls. FEC were investigated in all animals at the day of treatment (0) and at days 14 and 21 post-treatment (p.t). In parallel, L3 were cultured from pooled faeces from all animals in each group. DNA isolated from pooled L3 was analysed by a qPCR quantifying copies of the second internal transcribed spacer (ITS2) specific for *Ostertagia ostertagi* and *Cooperia oncophora*. FEC reduction percentages (FECR%) in IVM-treated groups were calculated following WAAVP guidelines including FECs of the control group. Reduced efficacy was defined when FECR% ≤ 95% and lower confidence interval [CI] ≤ 90%.

Results: At day 14 p.t., FECR% of IVM treatments in all six farms varied from 75% to 96% (lower CI ranging from 39% to 91%). At day 21 p.t., IVM treatments showed a reduced efficacy in all farms, with FECR% varying from 45% to 91% (lower CI ranging from -34% to 65%). At day 0, ITS2 copies from *O. ostertagi* and *C. oncophora* were indentified in larval cultures from treated groups in all six farms. At day 14 p.t. in treated animals, *O. ostertagi* L3 were found in 1 farm and *C. oncophora* L3 in 3 farms. On day 21 p.t., *O. ostertagi* L3 and *C. oncophora* L3 were detected in treated animals of 2 and 4 farms, respectively.

Discussion: Reduced field efficacy of IVM was confirmed by FECRT in 5 out of 6 farms at day 14 p.t. and in all farms at day 21 p.t. The qPCR was able to identify *O. ostertagi* and *C. oncophora* populations surviving IVM treatment. This emphasizes the need of monitoring the efficacy of anthelmintic treatments in cattle using sensitive methods.